REMARKS

Applicants thank the Examiner for withdrawing the previous rejection of the claims under 35 U.S.C. § 112 first paragraph.

Rejection Under 35 U.S.C. § 102

Claims 1, 2, 4, 6 and 8 were rejected under 35 U.S.C. § 102(b) as anticipated by Liebl, *et al.*, *J. Bacteriology* 174(6):1854-1861 (1992) ("Liebl"). Applicants respectfully traverse this rejection, and respectfully request withdrawal in of this rejection in view of the enclosed declaration under 37 C.F.R. § 1.132 executed by Professor Wolfgang Liebl (discussed below).

The Legal Standard

For a rejection of claims to be properly founded under 35 U.S.C. § 102, it must be established that a prior art reference discloses each and every element of the claims. *Hybritech Inc. v. Monoclonal Antibodies Inc.*, 231 U.S.P.Q. 81 (Fed. Cir. 1986); *Scripps Clinic & Research Found. v. Genentech Inc.*, 18 U.S.P.Q.2d 1001 (Fed. Cir. 1991), and it must enable a person skilled in the art to make and use the invention. "A claimed invention cannot be anticipated by a prior art reference if the allegedly anticipatory disclosures cited as prior art are not enabled". *Amgen, Inc. v. Hoechst Marion Roussel, Inc.*, 314 F.3d 1313, 1354, 65 U.S.P.Q.2d 1385, 1416 (Fed. Cir. 2003).

Analysis

Liebl does not recite all of the claim limitations and does not enable one of ordinary skill in the art to make the claimed bacteria.

The Examiner alleged that Liebl discloses the claimed bacterial strain from *E. coli*, at Table 1, in which Liebl teaches the propagation of ASO19/PWNuc5 in *E coli* and *C. glutamicum*. Office Action mailed March 12, 2009. However, that this is not tantamount to a disclosure of *E. coli*, genetically modified to *express* a heterologous nuclease gene, wherein the nuclease gene product is *secreted* into the periplasmic space and released when the bacteria is lysed. The *E. coli* was simply used as a host for plasmid propagation. See for example Liebl, page 1854 which states "the *Escherichia coli* strain used for plasmid construction was JM83". A careful review of the disclosure in Liebl does not indicate any data with respect to expression and secretion of SNase in *E. coli*. The claims require that the bacterial strains express the heterologous nuclease gene, and secrete it into the periplasmic space. There is no such disclosure in Liebl with respect to *E. coli*.

The Examiner's attention is drawn to the enclosed declaration under 37 C.F.R. § 1.132, executed by Prof. Wolfgang Liebl, the lead author of the Liebl reference. The declaration notes that no expression of the SNase enzyme is described in the Liebl reference with respect to *E. coli*. ("Liebl declaration") See especially, item 7 of the Liebl declaration. Thus, the Examiner's allegation that Liebl discloses clearly teaches the *E. coli* which expresses and secretes a nuclease into the periplasmic space is incorrect. Office Action mailed March 12, 2009, page 4, para. 4.

Liebl does not disclose the claimed bacterial strain selected from the group consisting of *Ralstonia eutropha, Pseudomonas putida* and *Escherichia coli*, genetically modified to express a nuclease gene which is secreted into the periplasmic space. Therefore, Liebl cannot anticipate the claims. Withdrawal of this rejection is respectfully requested.

With respect to claim 7, Liebl does not disclose an integrated gene, but a plasmid that requires induction for expression.

Thus, Liebl does not disclose all of the claim limitations as required for a rejection under 35 U.S.C. §102(b) and cannot anticipate the claims.

Therefore, claims 1-8 are not anticipated by Liebl.

Rejection Under 35 U.S.C. § 103

Claims 1-4 and 6-8 were rejected under 35 U.S.C. § 103(a) as obvious over WO 94/10289 by Greer, *et al.*, ("Greer"), Atkinson, *et al.*, Biochemical Engineering and Biotechnology Handbook, 2nd Edition, Stockton Press: New York, 1991 ("Atkinson") and Lee, *et al.*, *Adv. Biochem. Eng. Biotechnol.* 52:27-58 (1995) ("Lee"), or Miller, *et al.*, *J. Bacteriology* 169(8):3508-3514 (1987) ("Miller") in view of Liebl et al., *J. Bacteriology* 174(6):1854-1861 (1992) ("Liebl"), or Miller. Applicants respectfully traverse this rejection.

The Legal Standard

When applying 35 U.S.C. § 103, the following tenets of patent law must be adhered to:

(a) determining the scope and contents of the prior art;

(b) ascertaining the differences between the prior art and the claims in issue;

(c) resolving the level of ordinary skill in the pertinent art; and

(d) evaluating evidence of secondary consideration.

Graham v. John Deere, 383 U.S. 1, 17-18, 148 U.S.P.Q. 459, 467 (1966). These four factors are traditionally referred to as the Graham factors.

Obviousness is a legal conclusion. *See Graham v. John Deere Co.*, 383 U.S. 1, 17-18, 148 U.S.P.Q. 459 (1966). The *Graham* analysis was recently affirmed by the Supreme Court in *KSR Int'l Co. v. Teleflex, Inc.*, 127 S. Ct. 1727, 82 U.S.P.Q.2d 1385 (2007).

The obviousness analysis requires looking at the invention as a whole. "Focusing on the obviousness of substitutions and differences, instead of on the invention as a whole, is a legally improper way to simplify the often difficult determination of obviousness." *Gillette Co. v. S.C. Johnson & Sons, Inc.*, 919 F.2d 720, 724, 16 U.S.P.Q.2d 1923 (Fed. Cir. 1990); *see Hybritech Inc. v. Monoclonal Antibodies, Inc.*, 802 F.2d 1367, 1383, 231 U.S.P.Q. 81, 93 (Fed. Cir. 1986).

Hindsight analysis, such as picking and choosing from prior art references using the claimed invention as a template, has long been forbidden. *See, e.g., In re Fine*, 837 F.2d 1071, 1075 (Fed. Cir. 1988), which states that "One cannot use hindsight reconstruction to pick and choose among isolated disclosures on the prior art to deprecate the claimed invention." In *KSR*, the Court also warned against the use of hindsight analysis in making an obviousness determination. The Court stated, "A factfinder should be aware, of course, of the distortion caused by hindsight bias and must be cautious of arguments reliant upon *ex post* reasoning." (*KSR*, 127 S. Ct. at 1742, citing *Graham*, 383 U.S. at 36 (warning against a "temptation to read into the prior art the teachings of the invention in issue" and instructing courts to "guard against slipping into the use of hindsight" (quoting M*onroe Auto Equipment Co. v. Heckethorn Mfg. & Supply Co.*, 332 F.2d 406, 412, 141 U.S.P.Q. 549 (6th Cir. 1964))).

Analysis

The Scope and Content of the Prior Art

Greer

Greer describes the exogenous addition of peroxide to a cell culture. As stated in the Examples of Greer, and as stated as one of the problems addressed by the presently claimed invention, the exogenous addition of nucleases is generally known and too expensive to use for commodity fermentation products involving high cell density fermentations.

Liebl

Liebl describes the heterologous expression of a *Staphylococcus aureus* nuclease gene in *C. glutamicum* and the use of this transgenic system for investigating protein export in *C. glutamicum*, as discussed above.

Miller

Miller discloses the use of a *B. subtilis* secreted nuclease for investigating "the nature of the processing of the nuclease signal peptide". Miller further characterizes the secretion of nuclease and the processing of the signal peptide from the precursor protein in *B. subtilis*. Miller speculates that the *staphylococcal* nuclease and its gene may be very useful for the development of secretion vectors for foreign proteins.

Atkinson

Atkinson is a general review of biochemical and biotechnological methods and reagents.

Lee

Lee reports on production of PHAs in bacteria, and control of fermentation conditions.

The Differences Between the Prior Art and the Claims

A combination of Greer, Liebl, Miller, Atkinson and Lee does not recite all of the elements of the claims.

None of the references cited by the Examiner disclose or suggest the claimed bacterial strain. None of the references cited by the Examiner disclose expression and secretion of a nuclease into the periplasmic space of any gram negative bacteria. In making a determination as to what the art teaches one of ordinary skill, the Examiner must consider each of the five references cited as allegedly rendering the claims obvious, as a whole.

Liebl and Miller disclose genetically engineering the gram positive bacteria *C. glutamicum* and *B. subtilis* respectively, to secrete nuclease into the culture medium.

The Examiner improperly relied on Liebl as allegedly disclosing *E. coli* which expresses and secretes a nuclease into the periplasmic space. Office Action mailed March 12, 2009, page 7, para. 1. However, as noted in the Liebl declaration, expression and secretion of nuclease is not disclosed in Liebl in connection with *E. coli*.

There is no motivation to combine the references as the Examiner has done and even such a combination does not arrive at the claims

The Examiner alleged that one of ordinary skill in the art would have been motivated to express the *Staphylococcal aureus* nuclease as taught by Liebl or Miller so that this bacterial strain would produce and excrete the nuclease into the bacterial growth medium as part of the fermentation process, in order to reduce the amount of nucleic acids in the medium which results in downstream fermentation processes, as taught by Greer. Office Action mailed March 12,

2009, page 8, para. 2. Even if the Examiner were correct, the claims do not require that the bacteria be genetically modified to secrete nuclease into culture medium. The claims require that the bacteria be engineered to secrete the nuclease into the periplasmic space, where it remains, until cell lysis. The Examiner has articulated no reasons why one of ordinary skill in the art would modify the disclosure in any of Liebl, Miller or Greer to secret nuclease into the periplasmic space of bacteria. Applicants provide an elegant system for reducing viscosity in fermentation media whereby bacteria are engineered to express an effective amount of nuclease, which is sequestered in the periplasmic space where it is harmless to the cells, until it is released when needed, by cell lysis. There is nothing in any of the references cited by the Examiner which would lead one of ordinary skill to this limitation. Any conclusion otherwise would be based on impermissible hindsight analysis.

The Examiner's attention is drawn to *In re Kotzab*, 208 F.3d 1352, 54 USPQ2d 1308 (Fed. Cir. 2000).

> "In rejecting a patent's claims, the PTO "fell into the hindsight trap." The idea behind a limitation in the claims--to use a "single temperature sensor to control a plurality of flow control valves"--was a "technologically simple concept." With the concept in mind, the PTO found statements in the prior art that "in the abstract appeared to suggest the limitation," but it made no finding of a specific understanding within the knowledge of a skilled artisan that would motive one without no knowledge of the invention to make the limitation. The PTO's finding that a single reference taught the "single sensor" by stating that a single "system" could control multiple valves lacked substantial evidentiary support because the reference did not use "single system" as synonymous with a "single sensor."

Similarly here, the concept of genetically engineering secretion of nuclease into the periplasmic space where it is sequestered until it is released when desired, appears to be a

"technologically simple concept" after one reads the enabling disclosure by Applicants. With this concept in mind the Examiner has searched the prior art looking for statements that appear to suggest limitations in the claims. Release of nuclease into the culture medium is not release into the periplasmic space as required by the claims. Further, such release does not present the additional advantage of (i) sequestering the expressed nuclease until it is released when desired during the fermentation process, by cell lysis or (ii) protecting the cells from nuclease due to the sequestration. Nucleases are toxic to living cells. See for example Bernath, *J. Mol. Biol.*, 345(5):1015-26 (2005) a copy of which is attached. *See* especially page 1016 which states "all nucleases are toxic to cells". Thus, sequestration and controlled release of the nuclease is desirable. *See* also, Cooke, *J. Biotechnoilogy*, 101(3):299-239 (2003), which discusses the advantages of sequestering nuclease in the periplasmic space. See especially page 230, right col., para. 3.

The Examiner's allegation that upon lysis, the bacterial strain taught by Liebl will secrete the nuclease gene product into the periplasmic space is incorrect. Office Action mailed March 12, 2009, page 10. As discussed above, Liebl does not disclose expression of the SNase gene product in *E. coli.* All Liebl discloses is propagation of a plasmid containing the SNase gene. Thus if the *E. coli* in Liebl were lysed, the plasmid would be released, not the gene product. There is no basis for the assertion that cell lysis will lead to secretion of the gene product into the periplasmic space. With respect to the *C. glutamicum* disclosed in Liebl, is it unclear how the bacteria which are engineered to secrete nuclease into cell culture medium, would secrete the nuclease into the periplasmic space if lysed. *C. glutamicum* does not have a periplasmic space.

See Zuber, *J. Bacteriol.*, 190(16):5672-5680 (2008) (a copy of which is attached). See especially, Fig. Legend of Fig.1).

None of Atkinson or Lee makes up for the deficiencies in Greer, Liebl and Miller. Lee discloses the production of copolyesters in *Pseudomonas sp.* Lee does not disclose genetically modifying any bacteria for secretion of protein into the periplasmic space. Atkinson, a review of biochemical and biotechnological methods and reagents, similarly does not make up for this deficiency.

With respect to claim 7, none of the prior art discloses genetically modifying bacteria with the heterologous nuclease gene integrated into the chromosome, and the gene product secreted into the periplasmic space.

The cited art, alone or in combination does not provide an expectation of success in arriving at the claimed bacterial strains

At time of publication of Liebl or Miller, it was widely believed that gram positive bacteria did not have a periplasmic space (see Sakamoto, *et al.*, *Microbiology*, 147:2865-2871 (2001), submitted by Applicants with the amendment and response filed on October 24, 2007. Thus, neither Liebl nor Miller both of whom expressed genes in gram positive bacteria, were concerned with targeting genes into the periplasmic space. Therefore, contrary to the Examiner's allegations, neither Miller nor Liebl provides one with an expectation of success in expressing a nuclease in the periplasmic space as claimed. Office Action mailed March 12, 2009, page 9. Atkinson, a review of biochemical and biotechnological methods and reagents, similarly does not make up for this deficiency. Biotechnology tools have been around for a long

time; however, it is still not possible to obtain expression of genes in certain organisms or locations within the organism, or expression to desired levels in 2009, let alone 11 years ago (i.e., March 30, 1998, the earliest priority date of this application); *See* for example Makrides, *et al.*, *Microbiol. Rev.*, 60(3):512-538 (1996) ("Makrides", a copy of which is was attached to the Amendment and Response filed on September 14, 2009), which states for example, "in spite of the extensive knowledge on the genetics and molecular biology of *E. coli*, not every gene can be expressed efficiently in this system" (see page 512, right col.). The Examiner presented no evidence to support an allegation that as of March 30, 1998, availability of biotechnology tools made obvious successful expression of any and every gene, at the desired location and at the desired levels in a recombinant organism. Thus, a combination of references, all of which are concerned with addressing a different technical problem, does not provide one with an expectation of success in expressing a nuclease gene product in the periplasmic space as claimed, which is released upon lysis of the bacteria.

In Summary

The Examiner has not met the burden of establishing a prima facie case of obviousness. The Examiner has provided no reason why one of ordinary skill in the art would modify the combination of Liebl, Greer, Lee, Atkinson and Miller, to express a nuclease in the periplasmic space of bacteria as claimed. The combination of Miller, Liebl, Greer, Atkinson and Miller does not provide any expectation in expressing nuclease in E. coli as claimed. Thus, the art cannot render the claims obvious.

Evidence of secondary considerations

As the Court reiterated in *KSR v. Teleflex*, evidence of long standing need and of commercial success are both secondary indicia of non-obviousness. Secondary considerations to be considered include commercial success, long felt but unresolved needs, failure of others, unexpected results, properties not present in the prior art etc.

The claims provide a simple yet elegant mechanism to endogenously produce nuclease in genetically modified bacteria, which enables cost effective processing of nucleic acids released during fermentation processes, avoids any deleterious effects of the expressed nuclease on the cells, and provides external control of the release of the nuclease. This is accomplished by directing expression of the nuclease gene product to the periplasmic space. Such expression sequesters the nuclease gene product, thus protecting the cells from the nuclease until needed, and enables release of nuclease into the fermentation mediums when desired. None of the references cited by the Examiner, alone or in combination provide bacteria with the properties of the claimed bacteria.

With respect to claim 7, chromosomal integration of the heterologous nuclease and expression of nuclease to high levels (shown in Example 6) avoids the use of plasmids which are difficult and expensive to maintain in large scale fermentations, as well as the use of IPTG which is cost prohibitive and toxic (see Makrides, page 514, left col.) that has been necessary in plasmid-based expression systems in the prior art, in order to obtain appreciable expression/secretion of nuclease. Such cost effective strains are highly desirable. The claims

provide microbial strains which are cost effective for fermentation processes and can enable more profitable production of the products listed in polyhydroxyalknoates.

The prior art does not provide the combination of properties recited in the claims. Accordingly, claims 1-4 and 6-8 are not obvious over Greer, Aktinson, and Lee or Miller in view of Liebl or Miller.

Allowance of claims 1-4, 6-8, 11, 12, 14-16, 19 and 21 is respectfully solicited. Claims 11, 12, 14-16, 19 and 21 are related to claims 1-8 as product and process of use. Accordingly, no new search would be required should claims 1-4 and 6-8 be found to be allowable.

Respectfully submitted,

/Patrea L. Pabst/
Patrea L. Pabst
Reg. No. 31,284

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PABST PATENT GROUP LLP
1545 Peachtree Street
Suite 320
Atlanta, Georgia 30309
(404) 879-2151
(404) 879-2160 (Facsimile)